Inhibition of Coliphage Reproduction after Superinfection of Induced Lysogens

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ABSTRACT

BRENNER, DON J. (University of Washington, Seattle), AND NEAL B. GROMAN. Inhibition of coliphage reproduction after superinfection of induced lysogens. *J. Bacteriol.* **92**:1727-1734. 1966.—Purified preparations of phages λ and λ 112 inhibited lysis, phage reproduction, and endolysin synthesis by ultraviolet-induced strains of K-12 (λ) and K-12 (λ 112). Structural and partial functional integrity of phage was required for inhibition, and the role of phage deoxyribonucleic acid (DNA) in inhibition was demonstrated. Both ultraviolet-irradiated and host-modified lysates of λ were inhibitory, suggesting that replication of superinfecting phage DNA was not essential for inhibition. Using various combinations of superinfecting and resident phage, including λ , 434, 434hy, and C-mutant derivatives, we observed that all three C region cistrons played some role in inhibition. Of the three, the C₁ cistron was the most critical. With certain phage combinations, inhibition was observed even though the resident and superinfecting phage differed in immune specificity. Both the medium and the method of superinfection determined whether lysis or lysis inhibition would occur. These and other observations with K-12(λ)*thy*⁻ indicated that many factors influence the outcome of superinfection. The data are compatible with the view that superinfection inhibition is due to the establishment or re-establishment of phage repressor activity, with the result that replication of both superinfecting and resident phage is blocked.

The superinfection of induced lysogenic bacteria by homologous and heterologous phages has been studied by a number of investigators (7, 14, 15, 17). In the recent work most germane to the present study, Korn (11) superinfected induced *Escherichia coli* K-12(λ)*thy*⁻ with either λ or λ c during the period of thymine deprivation. Upon readdition of thymine, lysis occurred and phage was produced which was predominantly of the superinfecting type. In contrast to these results, we found that lysis, phage reproduction, and endolysin synthesis were inhibited after superinfection of ultraviolet (UV)-induced *E. coli* lysogens by either homologous or heterologous λ or 434 phages. The relationship of these inhibitory effects to the structural, functional, and genetic characteristics of phage is described.

MATERIALS AND METHODS

Bacteria and phages. Cultures of *E. coli* K-12, K-12Sr, K-12(λ), K-12(λ 112), K-12(λ c67), K-12

(λ c68), K-12(434) (phage from A. Campbell), K-12(434hy) (phage from A. Campbell), K-12C600, K-12C600(λ), K-12C600(434) (phage from A. D. Kaiser), K-12C600(434hy) (phage from A. D. Kaiser), *E. coli* CS (strain C from R. L. Sinsheimer), CS(λ), CS(λ 112), CSF⁺(λ), CSF⁺(λ 112), CT (strain C from I. Tessman), CT(λ), CT(λ 112), CTF⁺(λ), and CTF⁺(λ 112) were obtained from the Microbiology Department of the University of Washington. Cultures of K-12(434hy c67) and K-12(434hy c123) were obtained from A. D. Kaiser. K-12(λ)*thy*⁻ was obtained from D. Korn.

Phages λ , λ 112, λ vir, 434, and 434hy (from A. Campbell) were obtained from the Microbiology Department of the University of Washington. Phages λ ind⁻, λ c50, λ c67, λ c68, 434hy, and 434hy c67 were obtained from V. Bode. Phages 434, 434hy, 434hy c1, and 434hy c123 were obtained from A. D. Kaiser. Phages λ c50 and 434hy c1 are C₁ mutants, λ c67 is a C₂ mutant, and both λ c68 and λ c123 are C₂ mutants.

Phage λ c123 was obtained by recombining phages λ and 434hy c123, and phage 434hy c68 was obtained by recombining phage 434hy and λ c68. A modification of the procedure of Jacob and Wollman (8) was employed. Mixtures of phage pairs to be used in recombination were suspended in LB-1 medium (at a multiplicity of infection of 10 to 15)

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and were irradiated with UV for 90 sec at the same dosage employed for induction. A sample of log-phase C600 cells held at 0 C for 60 min was then sedimented and immediately resuspended in 6 ml of the phage mixtures in the dark (to prevent photoreactivation). The cultures were incubated for 20 min at 37 C, and then sedimented and drained well. They were then resuspended in LB-1 medium and incubated until lysis occurred. The lysates were chloroformed and cell debris was removed by centrifugation. Recombinants were isolated on the basis of immunity and plaque type. In both isolations, the lysate was initially plated on K-12Sr. Phages in individual, clear plaques were then tested for their response to λ or 434 immunity by use of K-12(λ) and K-12(434hy), respectively.

Media. TSY broth, which contained Trypticase Soy Broth (BBL) supplemented with 0.25% dipotassium phosphate and 1.0% yeast extract, was routinely used as growth medium.

LB broth contained 0.5% yeast extract, 1.0% tryptone (Difco), 0.1% glucose, and 1.0% sodium chloride at pH 7.4. LB-1 broth, routinely used as a post-induction medium, lacked glucose and contained 5×10^{-3} M magnesium sulfate. In the endolysin assay and on other occasions, glucose or sodium chloride, or both, were omitted from the medium.

Nutrient broth, containing 0.3% beef extract (Difco) and 0.5% peptone (Difco) at pH 7.0, was used in experiments involving effects of postinduction medium on lysis inhibition.

Solutions and reagents. Cesium chloride from the American Potash and Chemical Corp., Los Angeles, Calif., was used in density gradient centrifugation for phage purification.

The enzymes and inhibitors tested for their effect on lysis inhibition were used at the following final concentrations: deoxyribonuclease (twice crystallized) and ribonuclease (five times crystallized) from Nutritional Biochemicals Corp., Cleveland, Ohio, at 18 μ g/ml; crystalline egg white lysozyme from Armour Laboratories, Chicago, Ill., at 100 μ g/ml; trypsin (twice crystallized) from Worthington Biochemical Corp., Freehold, N.J., at 18 μ g/ml; pronase (B grade) from Calbiochem, Los Angeles, Calif., at 50 μ g/ml; sodium metaperiodate (reagent grade) from G. Frederick Smith Chemical Co., Columbus, Ohio, at 2×10^{-4} M.

General experimental conditions. Phage assays, chloroform lysis, UV induction of lysogenic strains, and endolysin assays were carried out as previously described by Groman and Suzuki (5, 6). All experiments were carried out at 37 C unless otherwise specified. Cultures were aerated on a reciprocal water bath shaker operating at 100 strokes/minute. Optical density was determined in a Klett-Summerson Colorimeter, with a no. 54 (green) filter.

Preparation of phage stocks. Log-phase cells of K-12 or C600 at a concentration of 5×10^8 per milliliter were centrifuged, and were then suspended in 10^{-2} M magnesium sulfate to a concentration of 2×10^8 cells per milliliter. A stock suspension of phage at an approximate multiplicity of infection of 5 was added. The culture was incubated for 15 min with

occasional shaking to allow phage adsorption. The culture was then diluted with an equal volume of double-strength LB (–glucose) or LB (–NaCl) and incubated until lysis was complete, after which a few drops of chloroform were added. The suspension was kept at room temperature for 20 min, during which time the cells were frequently shaken. Cell debris was removed by centrifugation and the lysate was stored at 4 C.

Phage stocks were obtained from lysogenic cultures by inducing the culture, diluting it into an equal volume of double-strength LB (–glucose), and incubating until lysis was complete. Subsequent treatment of induced lysates was identical to the treatment of lysates obtained by infection from without.

Superinfection experiments. Log-phase cells at a concentration of 5×10^8 cells per milliliter were sedimented, suspended in 10^{-2} M magnesium sulfate to a concentration of 2×10^8 per milliliter, and induced. Samples (3 ml) of the irradiated cells were transferred to screw-cap test tubes. The cells were then sedimented and resuspended in 6 ml of either a crude lysate or purified phage diluted with LB-1. In most experiments, no effort was made to remove superinfecting phage. In some experiments, superinfecting phage was removed after 5 to 15 min by centrifuging and washing the cells and then resuspending them in 6 ml of LB-1. Superinfecting purified phage was usually added directly to induced cells (preadsorbed) and allowed to adsorb for 5 to 15 min before the cells were sedimented and resuspended in LB-1.

The cultures were incubated and Klett readings taken at 30-min intervals. Phage assays were carried out at 120 min (or when desired). A culture was considered to be lysis-inhibited if slight or no reduction in optical density occurred between 60 and 120 min after induction. A culture was considered lysed when a pronounced reduction in optical density (at least 30 Klett units) was observed between 60 and 120 min after induction.

RESULTS

Effect of crude λ 112 lysates on the lysis of *E. coli* CT(λ 112). Groman (4) reported that induced K-12(λ 112) lysed at 37 C when sodium chloride was present in the postinduction medium and exhibited lysis-inhibition in the absence of this salt. The possibility that an inhibitor was produced in the medium in which lysis-inhibition occurred was investigated.

E. coli CT(λ 112) cells were induced and diluted with an equal volume of either double-strength LB (–glucose) or double-strength LB (–glucose, –NaCl). The cultures were incubated at 37 C for 4 hr, by which time the culture containing salt had lysed and the culture without it had entered lysis-inhibition. The cultures were centrifuged to remove bacterial debris, and then the supernatant fluids, together with fresh-media controls, were employed as postinduction media for newly induced CT(λ 112) cells. Figure 1 shows the usual

FIG. 1. Effect of crude $\lambda 112$ lysates on the lysis of induced *Escherichia coli* CT($\lambda 112$). The cells were induced and diluted 1:2 in the following postinduction media: ○ = LB (–glucose), △ = LB (–glucose, –NaCl), □ = crude $\lambda 112$ lysate produced in LB (–glucose), ● = crude $\lambda 112$ lysate produced in LB (–glucose, –NaCl).

patterns for the controls. In contrast, supernatant fluids from both lysis-inhibited and, unexpectedly, lysing cultures produced lysis-inhibition. In subsequent experiments, it was shown that inhibitor was produced by and acted upon strains of *E. coli* lysogenic for either λ or $\lambda 112$. Thus, lysis-inhibition under these conditions was distinct from that produced by $\lambda 112$ as a result of its mutation.

Preliminary experiments showed that phage and inhibitory activity were inseparable on the basis of sedimentation, heat stability, and resistance to degradation or alteration by deoxyribonuclease, ribonuclease, lysozyme, trypsin, pronase, and sodium *meta*-periodate. Since lysis-inhibition is mediated by phage in other systems (3, 7, 13), phage was purified and tested for inhibitory activity. Phage was purified from crude lysates of K-12(λ) and K-12($\lambda 112$) by Kaiser's procedure (10), which involves two rounds of high- and low-speed centrifugation followed by banding in cesium chloride.

Purified phage (ca. 3×10^9 per milliliter) was added to induced K-12($\lambda 112$), either before or after dilution out of $MgSO_4$ into various post-induction media. Twelve media were tested, and lysis-inhibition was observed in 18 of the 24 combinations (Table 1). The data, which are presented in semiquantitative form, are based on optical density measurements. They demonstrate

conclusively that phage was responsible for lysis-inhibition. The existence of six postinduction media in which significant lysis-inhibition was not observed indicated that in some cases some other factor was required for an inhibitory response. This aspect of the study will be considered later.

Effect of sonic treatment on inhibition. Preparations of λ phage containing ca. 10^{10} phage per milliliter were inactivated by sonic oscillation, to a final concentration of 2×10^6 per milliliter. These preparations were unable to inhibit lysis of induced K-12(λ) cultures.

Antiphage antibody and heat inactivation. Crude lysates of K-12($\lambda 112$) were treated with anti-phage antibody until a 4-log reduction in phage viability was achieved. This preparation, and a similar preparation treated with normal rabbit serum, were tested for their ability to inhibit induced K-12(λ). The normal serum control produced lysis-inhibition, but the antibody-treated preparation did not.

Heating a crude lysate of K-12($\lambda 112$) to 65 C for 10 min, during which time the viable count fell from 1.9×10^{10} to 4.9×10^8 , also destroyed the inhibitory activity of the preparation. In general, heated lysates containing 10^9 (or more) phage per milliliter were inhibitory, whereas lysates containing fewer than 5×10^8 phage per milliliter were no longer inhibitory.

Thus, antibody treatment and heating, both of which probably affect phage-adsorption sites, destroy the ability of phage to inhibit lysis.

UV-inactivated phage. Lysates of λ phage were irradiated for varying periods of time under the same conditions used for induction. Under these conditions, phage was inactivated by UV light at a rate of about 1 log/10 min. The irradiated samples were assayed for viable phage and were then used as postinduction media for freshly induced K-12(λ) cells. These lysates retained the ability to inhibit the lysis of induced cells even though a 3-log reduction in viability (from an initial concentration of 8.8×10^9 per milliliter) had occurred. Multiplicity reactivation appears unlikely in this system, but has not been ruled out.

Host-modified phage. Phage $\lambda 112$ from induced *E. coli* CT($\lambda 112$) is host-modified with respect to the restrictive host *E. coli* K-12, plating with an efficiency of approximately 10^{-4} . Host-modified phage at concentrations of about 10^6 per milliliter as assayed on K-12, inhibited lysis of induced K-12(λ) and K-12($\lambda 112$). It may be asked whether the irradiation used for induction did not increase the efficiency of reproduction of modified phage. Bertani and Weigle (2) observed that UV irradiation sufficient to inactivate

TABLE 1. *Effect of varying postinduction media on lysis-inhibition of induced K-12(λ I12) by purified λ phage*

Medium	Supplement		Degree of lysis ^a		
	NaCl	MgSO ₄ (5×10^{-3} M)	Phage preadsorbed	Phage added to postinduction medium	Control without phage
	%				
Nutrient.....	—	—	+	—	++++
Nutrient.....	0.5	—	+++	—	++++
Nutrient.....	—	+	—	—	++++
Nutrient.....	0.5	+	+	—	++++
TSY.....	Deleted ^b	—	—	+	++++
TSY.....	0.5	—	—	+++	++++
TSY.....	Deleted	+	—	—	++++
TSY.....	0.5	+	—	—	++++
LB.....	Deleted	—	+	—	++++
LB.....	1	—	+	—	++++
LB.....	Deleted	+	—	—	++++
LB.....	1	+	—	+++	++++
LB (—glucose).....	Deleted	—	+++	—	++++
LB (—glucose).....	1	—	+++	—	++++
LB (—glucose).....	Deleted	+	—	—	++++
LB (—glucose).....	1	+	—	+++	++++

^a Symbols: ++++ = normal control lysis; +++ = extensive lysis; + = slight lysis; — = lysis-inhibition.

^b Omitted from the original formula.

82% of a nonlysogenic restricted host population allowed modified λ to plate with a 30-fold increase in efficiency over that in unirradiated cells. It would have taken a 500-fold increase to restore the modified phage to a level of activity normally required for inhibition. It seems safe to conclude that modified phage does inhibit.

Production of infective phage. Strain K-12(λ) was induced; the cells were sedimented and suspended in either crude CT(λ I12) lysates or in LB-1. Phage assays were performed when the control culture had lysed. Lysis-inhibited cultures contained only about 10% of the phage present in the lysed cultures. These results were confirmed and extended in one-step growth experiments (Fig. 2) in which induced K-12(λ) cultures suspended in LB-1 or crude CT(λ I12) lysate were incubated and assayed for phage at 15-min intervals for a total of 120 min. Infectious-center determinations were carried out for the first 45 min of the latent period. Additional samples were treated with chloroform to lyse the cells and liberate mature phage. Samples treated with chloroform at 30 and 45 min contained less than 0.1 mature phage per cell, which indicated that the infectious-center count reflected the number of induced cells up to 45 min.

In addition to a 1-log reduction in final phage titer, only 10 to 15% of the cells in the inhibited cultures were induced, compared to approxi-

FIG. 2. One-step growth curve during lysis-inhibition of induced K-12(λ) by a λ I12 lysate.

mately 85% in the controls (based on comparative infectious-center values at 45 min). Since the earliest infectious-center values were identical in inhibited and control cultures, induction was seemingly aborted or reversed during the course

TABLE 2. *Inhibition of phage and endolysin synthesis in various postinduction media*^a

Medium	Endolysin inhibition	Phage inhibition
	%	%
TSY (-NaCl).....	33	45
TSY (-NaCl, +MgSO ₄).....	43	32
LB (-NaCl).....	0	0
LB (-NaCl, +MgSO ₄).....	25	71
LB (-glucose, -NaCl).....	32	26
LB (-glucose, -NaCl, +MgSO ₄).....	75	88

^a Purified phage λ was preadsorbed to induced K-12(λ), after which the superinfected cells were sedimented and then resuspended in the appropriate medium.

of inhibition. These data also indicate that the small percentage of inhibited cells undergoing induction produced a fairly normal phage yield, whereas the majority of the inhibited population did not produce any phage.

Endolysin production. The effect of inhibition on the production of endolysin, the phage-induced specific lytic enzyme, was studied. The results of a series of experiments in which different media were employed are given in Table 2. Endolysin production was indeed inhibited during lysis-inhibition, and the degree of inhibition closely paralleled phage inhibition.

Colony formation after inhibition. A small percentage of lysogenic cells retain their colony-forming ability after UV irradiation. If inhibition reversed the induction process, an increase in the number of colonies might be observed. This was tested by superinfecting induced K-12-1C(λ)C cultures with λ c50 or λ , and plating for colony-formers at intervals. Since phage was not removed, the only cells capable of forming colonies were lysogenic or resistant λ mutants.

The K-12-1C(λ)C culture contained 1.3×10^8 cells per milliliter before induction. Of the induced cells superinfected with λ , 7% formed colonies at 10 min (compared to 3% in the induced control and the induced culture superinfected by λ c50). When the cells were plated at 30 min, only 1.5% of the control cells formed colonies (compared to 8.6 and 7.7% in the cultures superinfected by λ and λ c50, respectively). Thus, superinfection does cause a significant increase in the number of colony-formers. The ability of all the cultures to form colonies diminished after 30 min. Preliminary observations indicate that the surviving clones retain their initial prophage. In a small fraction of the cells, either recombinant phage or double lysogeny was observed.

Relation of the C region to inhibition. The C

region of λ phage plays a major role in the establishment and maintenance of lysogeny as well as in immunity to superinfection. To test the relationship of C functions to inhibition, various C-region mutants and hybrids of λ and 434 phage were employed, both as resident and superinfecting phage. It should be recalled that λ and 434 differ in their C region as well as at other loci, but that 434hy contains only the C₁ cistron of 434, which determines immune specificity. The remainder of its genome is derived from λ .

In general, in these experiments, the lysogenic bacterial culture was induced, centrifuged, and then suspended in a crude phage lysate containing the superinfecting phage which had been produced in LB-1 medium. The lysates were normally diluted 2- to 10-fold in LB-1 to obtain a final concentration of 10^9 to 3×10^9 phage per milliliter; in some of the early experiments, however, undiluted lysates containing 5×10^9 to 1.5×10^{10} phage per milliliter were used. Phage was assayed 120 min after induction. Percentage inhibition was determined by comparing phage titers in superinfected cultures and nonsuperinfected control cultures in LB-1. The superinfection results are presented in Table 3.

Some general observations are the following.

(i) Superinfecting wild-type λ phage inhibited induced homologous phages. Mutation in the C₁, and in some instances the C₂, locus reduced inhibition in varying degrees, but did not abolish it. Mutation in the C₃ region left the phage as effective as the wild type. (ii) Superinfecting wild-type λ phage also inhibited induced heterologous phage 434 and 434hy, although there was some difference in the degree of inhibition of various derivatives of the latter. The C₁ locus was relatively more important to inhibition than the C₂ locus, whereas once again the C₃ region appeared to play no role in inhibition. (iii) Superinfecting wild-type 434 phage inhibited homologous 434 fairly well; it partially inhibited induced 434hy and some of its derivatives, but was unable to inhibit induced heterologous phage. (iv) Superinfecting 434hy inhibited induced 434 and 434hy phages. Mutations in the C region diminished this ability with C₁ (the most critical locus) and C₂ and C₃ (somewhat less critical). (v) Superinfecting 434hy also inhibited induced wild-type λ phage. Mutations in the C₁, C₂, or C₃ locus of the superinfecting phage or in the C₂ or C₃ locus of the resident λ phage abolished the ability of 434hy to inhibit. (vi) Lysis-inhibition usually accompanied inhibition of phage production when the latter was 60% or higher. The notable exception to this rule was the inability of λ ind⁻ to inhibit lysis in 434 and 434hy lysogens in spite of 74% or greater inhibition of phage production.

TABLE 3. *Effect of C-region alterations on superinfection inhibition^a*

Mutation	Avg inhibition (%) of phage production ^b								
	K-12-1C (λ)C	C600C (λ)c	K-12-1C (λ c67) K	K-12-1C (λ c68) K	K-12-1C (434) C	K-12C (434hy) C	C600C (434hy) K	K-12K (434hy c67)K	K-12K (434hy c123)K
λ C.....	18) 74	3) 87	4) 74	3) 95	4) 80	4) 24	3) 77	4) 81	4) 36
λ I12.....	10) 68								
λ c50B.....	9) 29	2) 37	5) 66	3) 75	4) 19	4) 20	2) 4	4) 5	4) 20
λ c67B.....	9) 72		3) 97	2) 94	5) 95	4) 92		4) 89	4) 96
λ c68B.....	9) 83		5) 56	4) 44	5) 44	5) 38		4) 15	4) 43
λ c123Br.....	4) 55	2) 55	3) 13	4) 43	1) 57		2) 54		
λ ind ⁻ B.....	9) 80				5) 74	4) 84		4) 77	4) 78
λ vir.....	4) 1								
434C.....	2) 8	3) 0	2) 0	1) 0	5) 61	4) 36	3) 25	4) 36	4) 51
434hyC and 434hyK.....	18) 57	3) 71	4) 12	3) 10	6) 99	4) 99	3) 82	4) 99	4) 99
434hy cIK.....	6) 12	1) 9	2) 0	1) 0	3) 22	4) 12	1) 17	4) 9	5) 18
434hy c67K.....	8) 10		2) 0	1) 0	5) 42	4) 39		4) 35	4) 20
434hy c68Br.....	3) 28	1) 9	2) 3	3) 19	1) 52	4) 36	3) 25		
434hy c123K.....	4) 14		2) 0	1) 0	5) 39	5) 42		4) 36	4) 40

^a The italic letter following the bacterial strain or phage designates its origin: B = Bode, Br = Brenner, C = Campbell, and K = Kaiser.

^b First figure in each column is the number of experiments. The data are given as the mean of the percentage inhibition of phage production.

The results of these superinfection experiments must be interpreted broadly and qualitatively, since the methodology employed in this survey was of necessity crude. There are, for example, unassessed variations due to the use of different bacterial strains as well as to the different phages employed. Differences in the rate of adsorption probably occurred. The high cell densities which were employed to permit optical density measurements could not be expected to yield precise data on phage production, owing to the readsorption of phage. Nevertheless, at the minimum the data are valid when comparing the results of the superinfection of a single bacterial strain with a series of closely related phages.

Effect of superinfection on induced K12(λ)thy⁻. Korn (11) observed that both superinfecting and resident phage were produced when K-12(λ)thy⁻ was superinfected with λ c or λ vir during the period of induction by thymine deprivation. The majority of the progeny were of the superinfecting phage type. Since we observed inhibition of both superinfecting and resident phage with phages similar to λ c, it was of interest to learn how his strain behaved under our conditions.

The effect of λ and λ I12 superinfection on strain K-12(λ) and K-12(λ)thy⁻ is shown in Table 4. The degree of phage and endolysin inhibition was similar for both strains. The optical density curves were virtually identical and reflected the degree of phage inhibition, i.e., partial inhibition of lysis with purified phage and complete

TABLE 4. *Phage and endolysin production in lysis-inhibited K-12(λ)thy⁻ and K-12(λ) cultures^a*

Strain	Treatment	Endo- lysin inhibi- tion	Phage inhibi- tion
		%	%
K-12(λ)	Phage λ pread- sorbed	49	64
K-12(λ)thy ⁻	Phage λ pread- sorbed	55	64
K-12(λ)	Crude CT(λ I12) lysate	100 ^b	97
K-12(λ)thy ⁻	Crude CT(λ I12) lysate	79 ^b	91

^a K-12(λ) and K-12(λ)thy⁻ were induced with UV in the usual manner and were either preincubated with λ phage for 10 min while suspended in MgSO₄ and then suspended in LB-1 medium or suspended directly in crude λ I12 lysate. Induced controls were either incubated for 10 min in MgSO₄ and then suspended in LB-1 medium or were immediately suspended in this medium.

^b Corrected for the endolysin present in the crude lysates.

inhibition with crude lysate. Thus, both strains behave similarly under the conditions of our experiments.

Unknown factor participating in lysis inhibition. It was previously shown (Table 1) that, in certain media and under certain conditions of infection,

purified λ phage failed to cause inhibition. Curiously, the same phage was effective when present in lysates produced on some of these same media. This finding suggested that, under some circumstances, some additional condition or factor might be involved in inhibition. In the course of this study we discovered conditions that converted a noninhibitory medium to one that was inhibitory. As seen in the last line of Table 1, purified λ phage did not inhibit when added to LB (-glucose) medium containing induced K-12(λ I12). However, if induced K-12(λ) was incubated in this medium until just before lysis time, then the supernatant fluids of these cultures were inhibitory when purified λ phage was added to a concentration of 2×10^9 per milliliter or higher. The preconditioned medium was not itself inhibitory and contained only 3×10^7 to 6×10^7 phage per milliliter prior to adding purified phage. All efforts to determine whether inhibition was facilitated by something produced during preconditioning were unsuccessful. The possibility that preconditioning facilitated superinfecting phage adsorption was also tested, and the data clearly showed that adsorption was greater in the unconditioned than in the preconditioned medium.

DISCUSSION

It has been shown in the present study that the induction of various K-12 lysogens carrying derivatives of either λ or 434 phages can be aborted by superinfection with either homologous or heterologous phages. In the most intensively studied case, the superinfection of K-12(λ) with λ phages, the reproduction of resident and superinfecting phage was inhibited, as was endolysin synthesis and lysis. A decrease in infective centers and a significant increase in colony counts was also observed. These results indicate that both resident and superinfecting phage were blocked before they could enter the vegetative state, a conclusion supported by the additional finding that the original prophage could be detected in cells that survived as colony-formers.

A number of experiments lead to the conclusions that phage structural integrity and some functional integrity are essential to inhibition, that phage deoxyribonucleic acid (DNA) is essential to the process, but that DNA replication and possibly DNA integrity are not. The need for structural and some functional integrity is supported by the observations that sonic-treated lysates and antibody- or heat-inactivated phage preparations were not inhibitory. The function of DNA is most directly supported by the differences in the degree of inhibition on superinfection with different C-region mutants. The existence of

media conditions in which superinfecting phage adsorbs but does not inhibit also lends indirect support to the function of DNA. The inhibitory action of UV-irradiated or host-modified phage suggests that phage DNA replication and perhaps integrity are not essential to the process, for it is unlikely that UV-irradiated phage DNA replicates (16) and it is likely that host-modified phage DNA is rapidly degraded (1).

The effects of C-region mutations on inhibition show that functions controlled by C loci participate in the process. Although involvement of other loci is not excluded, the data in Table 3 can be rationalized on the basis of C-region activity. This can be done by considering inhibition as the re-establishment of C_1 repressor activity (see reference 9 for general review of this theory), the process being nonspecifically enhanced by intact C_2 and C_3 loci.

In those cases where superinfecting and resident prophage are at least genetically homologous for C_1 , then inhibition could theoretically, though not necessarily, occur if a single intact C_1 cistron was present. In those cases where the phages involved were heterologous in the C_1 cistron, then inhibition could theoretically occur only if both C_1 cistrons were intact, but could not occur if the superinfecting phage was a C_1 mutant. These minimal predictions are fulfilled by the data in Table 3. Thus, a significant degree of inhibition was achieved when mutant C_1 was used to superinfect strains carrying phage with a homologous C_1 cistron, whereas superinfection with heterologous phages produced inhibition only in those cases in which both C_1 cistrons were intact.

We have assumed that the C_2 and C_3 functions are nonspecific. This view is supported by the fact that mutation in either cistron influenced infections in which both the superinfecting and resident phages were 434 hybrids. In these infections the C_2 and C_3 loci derived from λ were heterologous to the C_1 locus derived from 434. The data in Table 3 provide additional evidence that C_2 and C_3 function in inhibition in a variety of situations and with varying effects.

Although abortion of induction has been reported to follow superinfection of K-12(λ)*thy*⁻ with λ *ind*⁻, a unique noninducible mutant of λ (7, 12), it has not to our knowledge been observed with the ordinary λ phages which produce or retain sensitivity to wild-type repressor. Strain K-12(λ)*thy*⁻, obtained from Korn, was also inhibited under the present conditions of induction and superinfection, although he did not observe it on infecting with either of two λ c mutants during the period of induction by thymine deprivation (11). These results only serve to emphasize

that differences in media, method of induction, or perhaps method of superinfection can all influence the outcome.

We have shown that the medium employed or the method of superinfection can determine whether lysis or lysis-inhibition follows superinfection. For the time being we do not know how these factors or conditions affect the decision. Lanni (13) described a substance in the filtrates of sonically disrupted uninfected bacteria which facilitated lysis-inhibition by a mutant of T5. These circumstances bear a resemblance to the present findings. Our findings suggest that failure to achieve inhibition is due to an effect on some step subsequent to adsorption. Where adsorption occurred and inhibition was not observed, it is possible that superinfecting phage was unable to penetrate.

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